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Risk spreading, habitat selection and division of biomass in a submerged clonal plant: Responses to heterogeneous copper pollution

Xue Yan [a,b,*], Haowen Wang [b], Qingfeng Wang [a,*], Lars G. Rudstam [c]

[a] Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China
[b] College of Life Sciences, Wuhan University, Wuhan 430072, China
[c] Department of Natural Resources, Cornell University, Ithaca, NY 14850, USA

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ABSTRACT

Heterogeneity of contaminant-stress can be an important environmental factor for clonal plants. We focused on Cu transport among the clones, the foraging or fugitive behavior and biomass allocation of submerged plant, *Vallisneria natans* (Lour.) Hara, exposed to heterogeneous sediments. This study was carried out in aquatic mesocosms between March and September 2010. Cu accumulated in contaminated ramets was exported horizontally via stolons to other ramets in uncontaminated patches, and then transported both acropetally to leaves and basipetally to belowground structures. There was no indication that *V. natans* adopted morphological plasticity in response to heterogeneous contaminated habitat. In contrast to predictions, more biomass was allocated to belowground tissues in contaminated patches. We concluded that risk of Cu stress spread among submerged clones, and *V. natans* did not actively select habitat in contaminated patchy environment. Furthermore, *V. natans* adopted compensatory investments instead of division of labor to acquire nutrient and survive.

1. Introduction

Wetlands are amongst the ecosystems most severely threatened by heavy metal pollution resulting from human activities (van Griethuysen et al., 2003; Buggy and Tobin, 2008; Lambert and Davy, 2011.).

In order to predict the bioavailability of toxic metals in water, some models (FIAM, GSIM) have been developed. As an extension of the FIAM, the biotic ligand model (BLM) has been suggested as a useful construct for assessing bioaccumulation and toxicity of metals to aquatic organisms (a-BLM) and terrestrial organism (s-BLM and t-BLM) (USEPA, 2003; Di Toro et al., 2005; Antunes et al., 2006; Luo et al., 2008; Thakali et al., 2006a,b). In these models, the influences of environmental factors, such as organic carbon content, pH, hardness, other dissolved metals and temperature on free ion activity of toxic metals are studied. Individual plants, such as wheat, lettuce, tomato were usually chosen as test plants in BLM to evaluate metal bioaccumulation and phytotoxicity (Parker et al., 1998; Cheng and Allen, 2001; Luo et al., 2008; Rooney et al., 2006), and only a handful of researchers have dealt with the ecological and toxic response of clonal plants in population level to heavy metals.

In general, clonal growth in plants is characterized by the ability to naturally produce repetitive units (ramets) with the same genotype, which may be interconnected via stolons or rhizomes and constitute a clonal population (Saikkonen et al., 1998; Price and Marshall, 1999). Compared with non-clonal plants, ramets of clonal plant in favorable patches can make a proportionally larger investment in tissue associated with resource uptake, resulting in a specialization of ramets within the clone. This is in line with the economic principle of spatial division of labor (DoL, Stuefer et al., 1996; Ikegami et al., 2008). DoL may result in more efficient exploitation of resources and the sharing of these resources within the clonal plant (Stuefer et al., 1996; Alpert and Stuefer, 1997; Oborny et al., 2000; Chesson and Peterson, 2002). The transportation of resources between ramets has been interpreted as physiological integration which strongly enhances the fitness of clonal plants (Outridge and Hutchinson, 1990; Yu et al., 2001).

Another important aspect is heterogeneous pollution. Heterogeneity is a fundamental property of ecosystems; both abiotic and biotic environmental factors have non-uniform distributions in space and time (Slade and Hutchings, 1987; Hutchings and Wijesinghe, 1997; van Kleunen et al., 2001; Pan and Clay, 2002; van Zandt et al., 2003). Different types or concentrations of heavy metals are also distributed in soil/sediment even in very small scale (Salminen and Haimi, 1999; Roiloa and Retuerto, 2006). This heterogeneity presents a challenge to non-clonal plants because

* Corresponding authors.
E-mail addresses: yanxue@whu.edu.cn (X. Yan), qfwang@wbgcas.cn (Q. Wang).

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they cannot actively move to favorable patches. However, clonal plant species may take advantage of patchy habitats in heterogeneous contaminated condition through morphological plasticity, directional clonal growth, and physiological integration among connected clones. Therefore we predicted that the metal tolerance, morphological plasticity of individuals and trend-avoidance behavior of individual plants and clonal plants are different in heterogeneous contaminated habitat.

In this study, we tested the hypotheses of risk spreading, habitat selection and DoL of a submerged clonal plant *Vallisneria natans* (Lour.) Hara subjected to spatially homogeneous or heterogeneous Cu-contaminated sediments. Specifically, we aimed at answering the following research questions: 1) does the risk of toxic stress spread among submerged clones through their physical connection? 2) how is the population of *V. natans* offspring distributed in heterogeneous Cu-contaminated habitat and whether submerged macrophytes avoid contaminated patches actively by morphological plasticity or not? 3) whether DoL occur or not among clones of submerged plants exposed to heterogeneous contaminated sediment?

2. Materials and methods

2.1. The study species

Vallisneria natans is a stoloniferous, perennial submerged native plant, which dominates in most lakes and ponds of the Middle-Lower Reaches of the Yangtze River, China (Yan et al., 2006). It overwinters as a short vertical stem from which some rosette-type ribbon-like leaves develop in early spring. In addition, a turion sprout one to three parent ramets, each of which may give rise to one or more primary offspring ramets followed by secondary offspring ramets. *V. natans* ramets generally forms two types of modified stems simultaneously, stolons and rhizomes, which are homologous organs. The stolon is a green horizontal stem that produces new ramets at the end of the growth tip, while the rhizome is an underground stem that produces a turion in the sediment through terminal expansion at the late growth stage. All ramets are connected by stolons and these remain over the lifespan of the plant. *V. natans* provides food, shelter and substrate, and serve as nursery habitat for many commercially important fish species.

2.2. The experimental design

The experiment was carried out in the outdoor aquatic mesocosm system of Wuhan University, China, from 29 March 2010 to 26 September 2010. Seventy-two turions of *V. natans* of similar size (0.67 ± 0.03 g) were used in the study. Each turion contained two buds. All turions were collected from the same population in Liangzi Lake, Hubei province, China (30°32′ N, 114°21′ E). The soil *in situ* (pH 6.74, TOC 27.4 g kg^{-1}, TP 0.49 g kg^{-1}, TN 1.97 g kg^{-1}, TK 20.2 g kg^{-1}, available N 143 mg kg^{-1}, available P 4.5 mg kg^{-1}, Cu 61.2 mg kg^{-1}) was also collected.

Both homogeneous and heterogeneous contaminated habitats were simulated using seven wooden hexagonal cells filled with soil with different levels of heavy metal added (Fig. 1). All cells were 5 cm deep and the area of each cell was set to 335 cm^2 based on the average stolon length of *V. natans* (8.84 ± 0.27 cm, $n = 5859$) recorded in our previous experiments. Different concentrations of $CuSO_4$ stock solutions were added into the soil from the lake and mixed thoroughly. The Cu concentrations of soils measured by flame atomic absorption spectrophotometry (Soil and Plant Analysis Council Inc., 1999) after mixing was 127.7 mg kg^{-1} for the low Cu level treatment and 289.4 mg kg^{-1} for the high Cu level treatment. The soil from the lake was used as control. Three sets of cells group were homogeneous with either control soil from the lake (T_0), low Cu level soil (T_1), or high Cu level soil (T_2) in all cells. In the heterogeneous habitat (T_3), the center cell was filled with control soil and the six surrounding cells were divided into three parts, T_3-C, T_3-L, and T_3-H, being filled with control, low Cu level and high Cu level soil, respectively. The thin partitions between cells prevented roots from growing into adjacent patches, whereas the upper edges of the partitions were leveled with the soil surface, enabling stolons to grow unhampered from center cell to T_3-C, T_3-L and T_3-H. Three turions were planted in each central cell so that the primary offspring had an equal chance to grow in each of six directions. Finally, all cells groups with soil and turions were placed in a glass tank (100 × 100 × 100 cm) filled with 85 cm deep water. Each treatment was replicated three times.

2.3. Measurements

In order to assess the diffusion of Cu from soil to water, water Cu levels in all tanks were analyzed on 30 March, 14 May, 6 July and 26 September by Atomic Absorption Spectrometer (AAS, Perkin Elmer, AA800). Water Cu levels did not differ significantly among tanks (Fig. 2).

After nearly six months, the plant material in each tray was harvested. The epiphytes and sediments were carefully removed from the plant material using tap water. The numbers of ramets and turions in each patch were counted and the lengths of all stolons were measured. The dry weights of leaves, roots, stolons, rhizomes and turions in each patch were determined after drying at 80 °C for 24 h. The ratio of total dry biomass/number of ramets, turions dry biomass/number of turions and stolon dry biomass/stolon length were calculated as a measure of ramet size, turion size and stolon thickness, respectively. We also calculated relative biomass allocation to stolons, leaves, roots and turions (adding rhizomes) in different habitats. In order to investigate the growth direction of offspring ramets, we divided the ambient space around each ramet into six equal areas and ensured that stolons had an equal chance to grow in all six directions. We defined all stolons located in each of six areas as having the same growth direction. The frequencies of stolon location were recorded.

Cu concentrations of the five tissues of the plants (roots, leaves, stolons, turions and rhizomes), in the different homogeneous habitats (T_0, T_1 and T_2) and in all patches (center cell, T_3-C, T_3-L and T_3-H) of the heterogeneous habitat were determined by AAS according to the method of Soil and Plant Analysis Council Inc (1999). The proportion of Cu standing stocks were calculated according to the following formula: $P_i = (C_i \times B_i)/\Sigma(C_j \times B_j)$; C_i, B_i was average Cu content and average dry biomass of a certain tissue, respectively. $\Sigma(C_j \times B_j)$ means total Cu standing stocks in five tissues.

2.4. Data analysis

One-way analysis of variance (SPSS-procedure GLM) was employed to test the effects of contaminated treatment. Normal probability and homogeneity of variance were examined. Average values of all measured variables were compared among the four treatments and among the three patches by SNK test. Chi-square contingency (the nonparametric test) analysis was used to test the differences among six growing directions of rhizome in homogeneous habitats and each patch of heterogeneous habitat. The statistical program package SPSS 11.0 was used for all calculations.

3. Results

3.1. Cu concentrations and standing stock in plant tissues

The mean Cu concentrations in the various tissues of *V. natans* ramets are presented in Table 1. The highest Cu concentrations were observed in roots with values ranging from 4.2 to 42.3 folds greater than in other tissues. All tissues of control plants (T_0) except roots

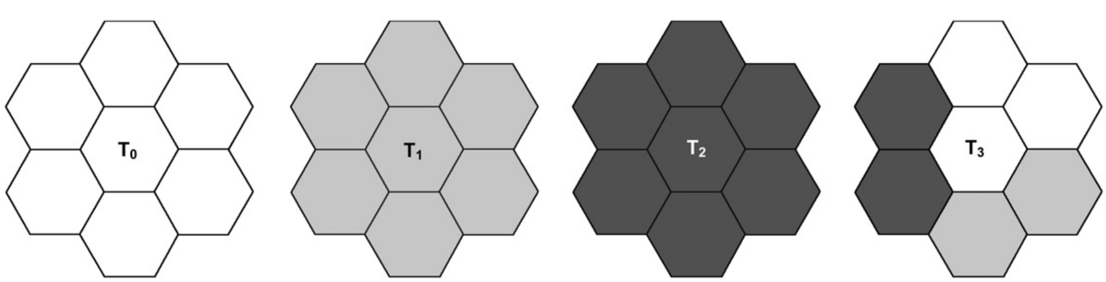

Fig. 1. The experimental design for one set of replicates. T_0–T_2 were the homogenous treatments, open hexagon: the control soil with natural background Cu content (61.2 mg/kg); gray hexagon: the soil with 127.7 mg/kg Cu; black hexagon: the soil with 289.4 mg/kg Cu; T_3 was the heterogeneous treatments, six surrounding patches were divided into three groups, T_3-C, T_3-L and T_3-H, filled with soil as in T_0, T_1 and T_2, respectively. The details of design were described in text 2.2.